either detectable changes in $[Ca^{2+}]_i$ or in barrier function which pointed to a complex role of Ca^{2+} signaling, if any, in endothelial barrier regulation. Absent or inconsistent $[Ca^{2+}]_i$ transients under conditions of increased cellular confluency further challenge a role of Ca^{2+}-mediated signaling in receptor-mediated disruption of barrier integrity. A thorough pharmacological examination revealed that different SOC inhibitors (e.g. lanthanides, 2-APB, BTP_2) completely abrogated store-operated calcium entry (SOCE) while having no effect on receptor-mediated disruption of endothelial barrier function thus suugesting that SOCE is not required for endothelial barrier regulation.

Cardiac, Smooth, and Skeletal Muscle Electrophysiology II

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Optimizing Rate Correction of Field Potential Duration, a Biomarker for QT Risk Assessment, in Human Ipsc-Cardiomyocytes

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The combination of human induced pluripotent stem cell-differentiated cardiomyocytes (hiPSC-CMs) with multi-electrode array (MEA) technology allows for a medium throughput assessment of cardiovascular risk at preclinical stages of drug discovery well in advance of candidate selection. Field potential duration (FPD) is a surrogate marker for QT duration and, like QT duration, is beat rate-dependent. Correction for changes in rate is required to properly interpret direct effects on FPD or QT. The FPD is defined as the time interval between initiation of the fast (Na^+ spike) voltage deflection due to I_{Na} and subsidence of the slow, positive deflection due to repolarizing I_K currents, and is a biomarker for QT duration. Our data demonstrate that FPD is highly correlated with the beat period in a largely non-linear fashion. Additionally, non-paced hiPSC-CMs are susceptible to dramatic drug-induced beat period changes. Consequently, interpretation of the effects of drugs on these parameters requires a beating rate correction. However, commonly used human ECG-derived QT correction formulae (e.g. Fredericia's, Bazett's) prove inadequate outside a narrow range of FPD-beat period linearity. Their use may incorrectly classify QT/FPD modifying compounds. We therefore developed and qualified a set of algorithms for FPD:beat period correction based on the response of hiPSC-CMs to the I_f inhibitor ZD7288 and the beta adrenergic agonist isoproterenol. The resulting FPD:beat period relationship had a limited linear range and an extended non-linear range. These algorithms were compared to other common correction methods. We found that individual correction provided a statistically significantly improvement over classical correction formulae (on parameters of linearity & slope) in the non-linear range. This correction algorithm has been incorporated into our MEA assay for QT risk assessment.

3648-Pos Board B376

Combine use of Intracellular Calcium and Sarcolemma Voltage Measurements to Distinguish Mixed Channel Effects in Human Induced Plutipotent Stem Cells Derived Cardiomyocytes (HIPSC-CMS)

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Blockade of the human ether-a-go-go-related gene (hERG) ion channel is a marker for pro-arrhythmic risk. However, the extent of hERG channel inhibition is poorly correlated with action potential duration (APD) prolongation *in vitro*, due to multi-channel block. Subsequently, compounds which block multiple ion channels may be overlooked by pre-clinical safety screening when measuring voltage alone, with the potential risk of late stage drug attrition and withdrawal. In this work we propose optical measurements of voltage and intracellular calcium ($[Ca^{2+}]_i$) in hiPSC-CMs as an alternative to the current drug screening methods to distinguish mixed action drugs in early stages. We used hiPSC-CMs loaded with 3μM di-4-ANEPPS to record membrane potential and 1μM Fura 4-AM to measure $[Ca^{2+}]_i$ from areas of iPSCs using CellOPTIQ platform (Clyde Biosciences, Glasgow UK). Fluorescence signals were digitized at 10kHz and analyzed off-line using proprietary software. Mixed ion channel blockade was mimicked by the co-administration of E-4031 (hERG blocker) and Nifedipine (Ca^{2+} channel blocker). The results showed that at a critical combined concentrations (e.g. 30nM E4031 + 30nM Nifedipine) which individually prolonged and shortened APD at 75% of repolarisation (APD75) respectively, in combination did not significantly change APD75 from baseline (APD75 6.5 ± 7% of control), whereas $[Ca^{2+}]_i$ was markedly reduced (by 18 ± 1.2% change, $p<0.05$). Low concentrations of Verapamil (30nM), an antiarrhythmic drug with known mixed hERG and L-type Ca^{2+} channel activity, had no significant effect on APD (APD75 1.5 ± 0.7%) whereas the amplitude of Ca^{2+} transient was significantly reduced (by 45 ± 8%, $p<0.05$). In conclusion, simultaneous measurements of $[Ca^{2+}]_i$ and voltage provides a convenient indicator of mixed ion channel effects when screening for drug-induced cardiotoxicity.

3649-Pos Board B377

Multiparametric One-Color Assays for Functional Assessment of Cardiomyocytes

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Heart-related health problems remain a leading cause of mortality in developed countries. To efficiently fight these problems, cardiology needs an information-rich screening for new drug candidates. Recent achievements in stem cell research provide a long-awaited solution by producing human stem-cell derived cardiomyocytes and, thus, providing various "disease-in-a-dish" models of cardiac disorders.

Two biologically distinct parameters are required to efficiently characterize the cardiomyocytes function: changes in cell membrane potential and distribution of intracellular calcium ions. Unfortunately, due to experimental limitations and lack of appropriate fluorescent indicators, the traditional approach to perform such an assessment is to record one signal at a time.

Here we present novel approach to perform multi-parametric assessment of cardiomyocytes while using two functionally different fluorescent indicators excited by the same wavelength of light. Specifically, using the IC200 HCS screening system (Vala Sciences), we performed a functional assessment of stem cell-derived cardiomyocytes by parallel optical recording of two physiologically distinct signals in real time: dynamic changes in cell membrane voltage as well as redistribution of calcium ions in cells. To do that, in addition to a calcium indicator Fluo4, we used a novel highly efficient voltage-sensitive fluorescent dye (VF 2.1) to detect voltage changes in cardiomyocytes.

To perform data analysis, we have developed a dedicated software program that by creating spatially distinct subsets of masks is separating calcium and voltage signals. Using our multiparametric assay, we have performed several screening campaigns using benchmark compounds such as modulators of ion channels involved in generation of cardiac action potential (hERG channels, sodium and calcium voltage-gated ion channels), as well as drugs that affect the intracellular calcium handling. In summary, we have developed a multi-parametric approach for comprehensive screening of a human stem cell-derived cardiomyocytes for drug discovery and cardiotoxicity purposes.

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A Novel Approach for Evaluation of Drug-Induced QT Prolongation using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Drug-induced cardiac arrhythmias characterized by QT prolongation have been a major reason for drug withdrawal at late stage of clinical trials. Species difference is a cause of insufficient predictability of current drug safety models. Therefore, human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) have great promise for application of cardiac drug safety testing as a human cardiac model. Our perforated patch-clamp recordings from hiPS-CMs revealed that the cells exhibited diverse shapes of spontaneous action potentials (APs) with relatively small upstroke velocity (~10V/s) and depolarized maximum diastolic potential (MDP; >-50 mV), which represents rather immature forms of cardiac cells. Actually, a selective hERG blocker, E4031,depolarized significantly MDP and stopped spontaneous beatings on a monolayer of hiPS-CMs, which makes it difficult to evaluate the effects on AP durations.Thus, in order to improve evaluation of risks for drug-induced QT prolongation in hiPS-CMs, we generated ventricular-like hiPS-CMs by over-expressing protein-X into hiPS-CMs and characterized the utility for evaluation of drug effects on cardiac repolarization process. The protein-X overexpression made hiPS-CMs quiescent with hyperpolarized MDP (−67 mV), and were excitable with rapid upstroke velocity (~95 V/s) by electrical field stimulation. When E4031 was applied to hiPS-CMs overexpressing protein-X, both AP durations of single myocytes and extracellular field potential durations of cardiac sheets were prolonged in a dose dependent manner. These results suggest that hiPS-CMs engineered with gene X would be a novel